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RESEARCH ARTICLE

Sympatric occurrence of three leaf beetle species of *Macroplea* Samouelle, 1819 (Coleoptera, Chrysomelidae, Donaciinae) in Finland with a key to species in Northern Europe

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Abstract

Macroplea Samouelle, 1819 is the only known fully aquatic leaf beetle genus with three European species that have earlier been classified by their assumed water salinity preferences. We studied the inter- and intraspecific variation of the specimens living in Northern Europe using both molecular (cytochrome *c* oxidase subunit I, COI) and morphological evidence. The variation in the COI sequences between *M. mutica* (Fabricius, 1792) and *M. pubipennis* (Reuter, 1875) was 8.4–9%, *M. mutica* and *M. appendiculata* (Panzer, 1794) 3.9–4.9% and *M. appendiculata* and *M. pubipennis* 8.8–9.2%. All three species were sampled together in the Bothnian Sea on the same water plants, showing that neither salinity nor plant species bear a decisive importance in their occurrence in the region. Phylogenetic results suggest the existence of two currently unknown *Macroplea* species that are evolutionarily close to *M. appendiculata*. A key to the Nordic species is provided.

Keywords

Coleoptera: Chrysomelidae: Donacinae: *Macroplea*, COI, phylogeny, key to species, northern Europe

Introduction

The leaf-beetle genus *Macroplea* Samouelle, 1819 exhibits one of the most extraordinary lifestyles in the family Chrysomelidae. All life stages from egg to adult live submerged, either in fresh or brackish water (e.g., Ljungberg, Lundberg and Wanntorp 2014). Northern Baltic members of the genus leave the water environment only by accident, possibly when birds or mammals move water plants from one spot to another or when loose water plants are left on shore by withdrawing water. *Macroplea* larvae use a pair of terminal hooks to attach themselves to the roots/basal parts of the stem of various water plants. These hollow hooks are modified spiracular atria that allow direct gas exchange between the larvae and the plant (Nilsson 1996). *Macroplea* cocoons are constructed around the full-grown larvae forming a shelter for the pupae (Owen and Menzies 1996). Adults hatch inside the cocoon and, at least in the waters of the northern Baltic, they stay in the cocoon for the winter leaving it the following spring/summer. Accordingly, the life cycle of *Macroplea* seems to last at least two warm periods (two summers) in the northern Baltic. Despite having a 100% aquatic life cycle, they are not regarded true aquatic insects because all their life stages are dependent on atmospheric air. The larva and pupa receive oxygen from the plant they are attached to but the adult carries an extensive plastron; a thin film of air attached to the body, which gets oxygen from the surrounding water (Thorpe and Crisp 1949). Adults also use the antennae for respiration (Nilsson 1996). During summer the adults crawl on water plants or on the bottom at depths of between 20–30 cm to 1.5 m or more. Feeding is scarce in adult *Macroplea*, according to Kölsch and Kubiak (2011), and limited to *Potamogeton* species. Even though adult beetles can also occupy, and reproduce on, *Myriophyllum* species, they do not use the plant as a food source. This might be due to insect growth and development inhibiting phenol compounds that *Myriophyllum* species are known to contain (Choi, Bareiss, Walenciak, and Gross 2002). Adult beetles

are active at least during daytime and in sunny weather; movements during dark have not been studied as far as we know (Solem 1972).

In total, the genus *Macroplea* numbers six Palearctic species. Three of them occur in Europe, viz. *M. mutica* (Fabricius, 1792), *M. appendiculata* (Panzer, 1794) and *M. pubipennis* (Reuter, 1875). All three species are present in countries of the Baltic region. As well as from Europe, all three species are today also known from East Asia, but interestingly *M. pubipennis* is only known from Finland and some Chinese provinces (Gansu, Heilongjiang, Ningxia, Xinjiang) (Kölsch, Biström, and Vest Pedersen 2006; Lou, Yu, and Liang 2011). Globally *Macroplea* is restricted to the Palearctic. Besides the three species discussed above, three more species are today attributed to the genus, namely *M. japana* (Jacoby, 1885), *M. ranina* Lou and Yu, 2011 and *M. huaxiensis* Lou and Liang, 2011. At least thus far, these three species are only known from Far East. Lou et al. (2011) discussed the taxonomy of *M. skomorokhovi* Medvedev, 2006 stating that the species is a junior synonym of *M. japana* although not officially published in a scientific journal thus far. For the time being, we treat this statement as a kind of synonymization.

The three species were earlier classified according to the salinity of the water they live in (Freude, Harde, and Lohse 1966; Mohr 1985). Thus, *M. mutica* and *M. pubipennis* were associated with brackish water and *M. appendiculata* with fresh water. Recently, doubts have been raised about this division because of some records of coexistence of *M. mutica* and *M. appendiculata* in both fresh and brackish water from the Baltic region (Kölsch, Krause, Goetz, and Plagmann 2010, Kölsch and Kubiak 2011). An extensive survey of *Macroplea* in fresh waters in the region of Lake Mälaren in Central Sweden was undertaken by Ljungberg et al.(2014). This study clearly showed that *M. appendiculata* and *M. mutica* both live in fresh water in Sweden. Salinity seems not to be a decisive factor for the presence of the two species, which was shown by Kölsch et al. (2010) who in laboratory preference tests concluded that both species prefer fresh water (salinity 0) to brackish water. The occurrence of the species may be influenced by other factors such as plant preference or mechanisms in dispersal, salinity playing a less important role. Kölsch and Kubiak (2011) compared the use of host plants by *M. mutica* and *M. appendiculata* and found little difference between species. Both *M. mutica* and *M. appediculata* preferred *Stuckenia pectinata* (L.) Börner */Potamogeton perfoliatus* L. to *Myriophyllum spicatum* L. as adults as well as during larval and cocoon stages. Only *M. appendiculata* used *Myriophyllum spicatum* for oviposition. Furthermore, *S. pectinata* was

solely used as a food source for adult individuals of both species. *Macroplea pubipennis* was not included in their study.

Most adults collected in the field are found in pairs, male clinging to a female, but the actual copulation is seldom performed and recorded. When the sexes meet they seem to approach each other and remain combined for longer time periods (Kölsch and Kubiak 2011). A plausible interpretation of this behaviour is as a method for the male to hinder copulation of the female by other males (e.g., Harari, Landolt, O'Brien, and Brockmann 2003). A common technique for encountering adult specimens in the field is to examine water plants, especially the top parts which are closest to the surface. During sunny weather conditions and reasonably calm water numerous adults can be obtained in this way.

Macroplea species are found on various water plants, but especially in the case of *M. pubipennis*, it has remained unclear which plants are preferred for development. Earlier information on living habits was mostly at genus level with little exact data on species (e.g., Biström 1996, Biström and Saari 2006, Lou et al. 2011, Ljungberg et al. 2014; Kölsch and Kubiak 2011).

Papers dealing with the taxonomy of *Macroplea* are comparatively scarce (e.g., Hoinic 1994, Kölsch et al. 2006, Medvedev 2006). There is a key available to identify adults (Lou et al. 2011), but we have encountered problems in some cases regarding the species pair *M. mutica* and *M. appendiculata*. Moreover the treatment of *M. pubipennis* is incomplete, lacking male genitalia illustrations.

The aim of this paper is to study the evolutionary relationships of the three North European *Macroplea* species and their inter- and intraspecific variation using both molecular and morphological evidence. We wish to clarify whether salinity plays a role in their distribution and whether host plant preferences differ between the species. Moreover, we give some additional characters for the species *M. pubipennis*, e.g., the appearance of the male genitalia not illustrated in Lou et al. (2011). The key presented here is constructed solely on the basis of adult specimens from the Baltic region.

Material and methods

Collecting of the material

The material was collected from several coastal localities of the Baltic Sea close to Finland (the Archipelago Sea and Bothnian Sea) and Estonia (Gulf of Riga) in June–August during the years 2001–2015 (see Figure 1 and Table 1). Some freshwater samples were additionally collected from Lake Puruvesi, which is part of Lake Saimaa in northern Karelia. Collecting methods included snorkelling, scuba diving, wading in shallow water and using motor-, rowing, or rubber boats. The first set of samples contained only adult specimens and was collected by one of the authors (Biström) during the years 2001–2015. This set was collected both by snorkelling (collecting specimens observed on the bottom and on host plants) and by wading in shallow water while inspecting the water plants with an underwater viewer (in sunny weather the adults are quite easily observed due to the plastron-air bubble around the body, which reflects a silvery glimmer). Suitable host plants were also looked for using a small floating vehicle (e.g., a rubber boat). When a stand of a host plant was located, some plant individuals were loosened from the bottom and inspected for *Macroplea* individuals in a floating, pale plastic tray (Figure 2).

The second set of samples was collected in 2015 by two of the authors (Kiviluoto and Laaksonen). All sampling sites were located in shallow sheltered bays (except Viasvesi Bay, which is an open bay) with abundant underwater vegetation, dominated mostly by *Stuckenia pectinata*. The bays were accessed by small motor- or rowing boats and vegetation densities for dominant species were estimated for each location. Both vegetation and beetles were studied either directly from the boat or by snorkeling. At deeper sites beetles were collected by scuba diving. However, since this direct method of collecting the beetles only provides adult individuals, potential host plants were also pulled up and studied in the boat in pursuit of additional adults, cocoons and larvae. All adult individuals were preliminarily identified to species level either during the day in the field or later in the laboratory. Most adult individuals were also photographed during the identification process.

All specimens were preserved in > 96% ethanol and kept in a freezer for DNA sequencing. The voucher specimens obtained from the first and second sets are held in the collections of the Finnish Museum of Natural History, University of Helsinki, and the Zoological Museum, University of Turku, respectively (see Table 1).

Laboratory methods

COI was amplified directly from the sample using Phire Tissue Direct PCR Master Mix (Thermo Scientific) using the Dilution and Storage protocol. Each sample was placed in 30 µl of Dilution Buffer into which 0.8 µl of DNARelease Additive was added. The reaction was first incubated at room temperature for 30 min and then at 98°C for 2 min. For each PCR reaction, 12 µl of purified water, 10 µl of 2X Phire Tissue Direct PCR Master Mix (containing gel loading dye), 1 µl of both primers and 1 µl of the supernatant were mixed. The COI primers used were LCO1490/HCO2198 (Folmer, Black, Hoeh, Lutz, and Vrijenhoek 1994) with a universal primer tail (T7Promoter(LCO1490) 5' TAA TAC GAC TCA CTA TAG GG 3' and T3(HCO2198) 5' ATT AAC CCT CAC TAA AGG G 3') attached. The PCR cycling protocol consisted of initial denaturation at 98°C for 5 min, followed by 40 cycles of denaturation at 98°C for 5 s, annealing at 49°C for 5 s and extension at 72°C for 20 s. Final extension was performed at 72°C for 1 min. A negative control was included in every PCR setup. PCR products were analysed by electrophoresis in 1% Agarose TBE and purified with the A'SAP PCR clean up kit (ArcticZymes). Purified samples were sequenced at Macrogen Europe. Chromatograms were visualised and assembled using the software Sequencher 5 (Gene codes corporation, USA). All sequences are deposited in GenBank (see Table 1 for accession numbers).

Phylogenetic analysis

The final molecular data consisted of 658 characters and 208 taxa. Two outgroups, *Donacia aquatica* (Linnaeus, 1758) (KJ966869) and *D. vulgaris* Zschach, 1788 (KM440282), were used. Phylogenetic analyses were conducted using both parsimony and likelihood approaches. Parsimony analysis was conducted in TNT v1.1 (Goloboff, Farris, and Nixon 2008) using the New Technology search with Sectorial search, Ratchet and Tree fusing. The analysis was run with default parameters except that the consensus was set to stabilise at 10 with a factor of 80. The jackknife (Farris, Albert, Källersjö, Lipscomb, and Kluge 1996)

resampling method was used to estimate nodal support (1000 replicates; probability of character removal = 0.36). Likelihood analysis was performed with RAxML v8.2.8. (Stamatakis 2014) in the CIPRES portal (Miller, Pfeiffer, and Schwartz 2010). Nodal support was estimated with the rapid bootstrap algorithm (1000 replicates). Uncorrected p-distances were calculated with MEGA v7.0.21 (Kumar, Stecher, and Tamura 2016).

Results

A total of 208 specimens were collected and of these 77 were larvae/cocoons and 131 adults (see Table 1 for detailed information). The TNT analysis resulted in 20 most parsimonious trees of length 307. The strict consensus of these trees, also showing the branch lengths, is shown in Figure 3. The parsimony tree divides the specimens into three strongly supported clades, the most basal division being between *M. pubipennis* (JF [jackknife] = 100), followed by *M. mutica* and *M. appendiculata* (JF = 99). The topology resulting from the likelihood analysis (ln*L* = -2300.302688, tree not shown) is congruent with that of parsimony with the exception of the basalmost species being *M. mutica*. Although collected from various localities (Figure 1), the amount of intraspecific variation within *M. mutica* and *M. pubipennis* specimens appears to be minimal (see Table 2 for pairwise distances). However, the specimens morphologically identified as *M. appendiculata* form three well-supported groups, one group being 99–100% identical to the *M. appendiculata* sequences available in GenBank and BOLD but two groups (*M. appendiculata* 2 and 3 in Figure 3) showing only 92–93% similarity. Unfortunately, only one specimen (no. 187) of the aberrant *M. appendiculata* groups was adult. This adult male was inspected morphologically but neither its genitalia nor its external habitus differed from the other adult *M. appendiculata* specimens in this study, all undisputedly identified as *M. appendiculata* using the key by Lou et al. 2011 (these are referred to as 'typical' *M. appendiculata* from here on).

All *M. appendiculata* specimens (incl. the two separate forms) were collected in the same brackish water localities where the two other *Macroplea* species also live, hence showing its ability to also live in brackish water with low salinity.

Intra- and interspecific variation

Pairwise distances between species are listed in Table 2. Within-species variation between *M. pubipennis* specimens is almost non-existent (< 0.2%). The low level of intraspecific variation also applies to *M. mutica* specimens (< 0.8%). The 'typical' *M. appendiculata* (*M. appendiculata* 1) sequences vary < 0.4% between the specimens, the sequences of the "species 2" *M. appendiculata* (two specimens) are identical and the sequences of the "species 3" *M. appendiculata* (four specimens) vary < 0.2%.

Interspecific variation between the *M. pubipennis* and *M. mutica* is 8.4–9%, *M. pubipennis* and "typical" *M. appendiculata* 10.2–11%. *Macroplea mutica* differs from the "typical" *M. appendiculata* by 3.9–4.9%, which is less than the amount the typical" *M. appendiculata* (1) differs from the two other *M. appendiculata* forms; *M. appendiculata* (1) differs from "*M. appendiculata* 2" by 4.9–5.3% and from *M. appendiculata* 3 by 6.7–7.1% (see Table 2).

Key to species of *Macroplea* in the northern Baltic region (adults)

1 Pronotum and elytra pubescent, with conspicuous longer hairs mixed with short hairs; apical spine of elytron short, broad at base (Figures 4a, 7a, 8a); pronotum pale, no dark spots on disc (Figure 6a); two basal metatarsomeres short and broad (Figure 5a); tip of penis apex minute, hardly discernible (Figure 9a); lateral aspect of penis (Figure 10a) ..... ***M. pubipennis***

- Pronotum and elytra glabrous, with shorter and sparser hairs (no clear mix of longer and sparser hairs); apical spine of elytron slender, narrower at base, length somewhat variable (Figures 4b-f; 7b-c); pronotum generally with two black spots (sometimes delimitation of spots vague) (Figures 6c-d, 7b); two basal metatarsomeres longer and more slender (Figures 5b-e); penis shape different; tip of penis apex distinct (Figures 9b-c, 10b-c) **2**

2 Apical spine of elytron generally longer, more slender (Figures 4d-f, 7b, 8b); second metatarsomere slightly longer than basal metatarsomere (Figures 5d-e); penis relatively robust, with distinct apical tip (Figures 9b, 10b) ..... ***M. appendiculata***

- Apical spine of elytron generally shorter and broader at base (Figures 4b-c, 7c, 8c); two basal metatarsomeres equal in length (Figures 5b-c); penis in dorsal aspect more delicate, almost parallel, apical tip hardly discernible (Figures 9c, 10c) ..... ***M. mutica***

Host plants

Host plant information was not obtained for all specimens in the dataset. However, for a total of 143 (out of 208) specimens, this information was available (Tables 1 and 3). Of these, most specimens (104 spp.) were collected from *Stuckenia pectinata*. *Macroplea appendiculata* specimens collected from *S. pectinata* were mostly adults (20 vs. three larvae), whereas *M. mutica* specimens included both adults (37) and larvae (42). The rarest species, *M. pubipennis*, included two adult and two larvae collected from *S. pectinata*. The second largest number of specimens (32 spp.) was collected from *Myriophyllum* species (*M. spicatum*, *M. sibiricum* and *Myriophyllum* sp.) Other plants from which small numbers of specimens were collected included *Potamogeton perfoliatus* (two spp.), *Ranunculus* sp. (two spp.), *Ruppia* sp. (two spp.) and *Zannichellia* sp. (one sp.).

Discussion

This study confirms that all three *Macroplea* species co-exist in coastal areas of Finland in the Bothnian Sea and that the species pair *M. mutica* and *M. appendiculata* may occur together at same site in brackish water where salinity is comparatively low (on average 0.5% in the southern part of the Bothnian Bay). The amount of fresh water from rivers running into the bay is large but varies, mainly due to differences in the yearly precipitation at a regional scale. This effect is especially clear in areas close to river mouths where salinity can be lower, sometimes almost zero. Our results are in line with those from Sweden (Ljungberg et al. 2014)

where no clear division in salinity preference between the two species was found, both species occurring in fresh water.

Distribution and dispersion of Macroplea pubipennis (Reuter, 1875)

Although all three *Macroplea* species exhibit a scattered Palearctic distribution, *M. pubipennis* has the most peculiar distribution: the species is found only in Finland and some Chinese provinces but not in between these areas. Mitochondrial COI sequences of *M. pubipennis* specimens from both countries have previously been analyzed phylogenetically (Kölsch et al. 2006) and found to be located in the same clade, within the limits of one species (the difference between the Finnish and Chinese specimens was < 0.3 %). There is no evidence thus far, for existence of two different species. Due to its low potential for active dispersion resulting from its lack of flying capability, past colonisation by *M. pubipennis* may have involved passive transportation. One possible way of passive transportation of *M. pubipennis* and other *Macroplea* species could have involved dispersion as egg stages inside the digestive tract of birds. There is supporting evidence for this, since a previous study showed that eggs of *M. mutica* are capable of viably passing through the digestive system of mallards *Anas platyrhynchos* Linnaeus, 1758 (Laux and Kölsch 2014). Also, mute swan *Cygnus olor* (Gmelin, 1789) movements have been found to be better predictors for the genetic structure of *M. mutica* than geographic distance (Laux 2014). Consequently, the intriguing distribution pattern of *M. pubipennis* in particular might indicate a rare case of zoochory.

Our study shows that *M. pubipennis* occurs at the same sites as the two other *Macroplea* species in brackish water of the Bothnian Bay (Baltic Sea). *Macroplea pubipennis* seems to be dependent on brackish water and avoid fresh water since there are no records of the species from freshwater bodies in Finland. Worth mentioning, however, is that *M. pubipennis* has been collected from sites with a huge input of fresh water which makes the salinity of the waterbody variable and partly low (Biström and Saari 2006). Why *M. pubipennis* solely occurs in brackish water in Finland remains unknown. Considering the Baltic Sea as a whole, we can only guess why *M. pubipennis* has not been recorded from Estonia and Sweden despite intensive, recent collecting activities along their coasts. It seems peculiar that the species could have arrived

at the Finnish coast and yet not spread to the neighbouring waters. Most likely the species will soon also be discovered from Sweden, Russia and Estonia when sampling activity is further intensified.

Notes on Macroplea appendiculata (Panzer, 1794)

The molecular results suggest the existence of two independent evolutionary lineages within *Macroplea* which differ from the three hitherto known species. Unfortunately, all but one specimen belonging to these two "mystery clades" (*M. appendiculata* 2 and 3 in Figure 3) were larvae, leaving the morphological comparison incomplete. On closer examination, the habitus of the single available male (no. 187) proved to be morphologically similar to typical *M. appendiculata* and its genitalia showed no difference from that of *M. appendiculata* (Figures 8b, 9b). Since no morphological (or biological) differences between the two new putative species and *M. appendiculata* were found, it is clear that more adult specimens need to be examined morphologically before new species can be described.

Plant preferences

With the introduction of molecular level analyses, new light is shed on the host plant relations of the three species. DNA sequencing enables us for the first time to identify larvae to species with almost 100% reliability. Earlier, adult specimens were sampled from various water plants but the correct combination of beetle species and plant only became available if newly hatched adults were acquired from the cocoons attached to plants. Sampling is still quite limited in our study, which restricts our chance to do exact statistical analyses of host plant-beetle preferences. In accordance with Kölsch and Kubiak (2011), our study shows that *Stuckenia pectinata* is the most favoured plant followed by *Myriophyllum* species. All three *Macroplea* species can, at least in the Bothnian Bay, use these two plant species for their development to adult.

The vegetation cover in shallow, sheltered bays preferred by *Macroplea* beetles can change both temporally and spatially (Munsterhjelm 1997; Pitkänen, Peuraniemi, Westerbom, Kilpi, and von Numers 2013; Hansen and Snickars 2014). The abundance of dominating plant species may vary over years, and the abundance of species that better tolerate eutrophication is likely to increase in the future (Pitkänen et al. 2013; Hansen and Snickars 2014). This may affect the survival of *Macroplea* species. Even though *Macroplea* species appear to prefer *Potamogeton* spp. (including *Stuckenia* spp.), they can survive and reproduce on several other species as well. *Macroplea* beetles are slow to recruit to new areas and, according to Kölsch and Kubiak (2011), they choose any submerged plant over bare bottom; it is, thus, likely that *Macroplea* populations will remain in bays with less preferred vegetation for at least as long as the plant coverage is abundant enough for the beetles to reproduce. Our results, in accordance with Kölsch and Kubiak (2011), suggest that during the short adult period of *Macroplea* reproduction is of more importance to the individuals than food intake.

Possible reasons causing and maintaining sympatric occurrence

Since we found no evidence of the three species (*M. mutica, M. pubipennis, M. appendiculata*) preferring different levels of salinity, habitats or host plants, the distribution pattern of *M. pubipennis* in particular may reflect passive transportation history more than active preference for certain habitats. In the case of *M. pubipennis*, immigrant inviability may additionally have contributed by producing reproductive isolation of the species (Nosil, Vines, and Funk 2005). This isolation is clearly shown in the phylogenetic COI tree (Figure 3) where *M. pubipennis* forms a distinct evolutionary lineage with up to 12% difference to the other *Macroplea* species. The amount of interspecific difference between *M. pubipennis* and *M. mutica* specimens is 8.4–9% and *M. pubipennis* and the "typical" *M. appendiculata* (*M. appendiculata* 1) 8.8–9.2%. The difference between *M. mutica* and *M. appendiculata* 1 is 3.9–4.9%. Intraspecific variation within these species is < 0.8% and < 0.4%, respectively. Differences between the three *M. appendiculata* forms were also clear; the *M. appendiculata* that we assume is the "typical" and most common form (*M. appendiculata* 1) differs from "*M. appendiculata* 2" by 4.9–5.3% and from "*M. appendiculata* 3" by 6.7–7.1%. All these

differences between species indicate strong positive assortative mating, i.e., sexual isolation between the species. Ecological population divergence and reproductive isolation between these species may have originated from several pre- and post-mating isolation factors. In previous research on insects, pre-mating isolation has been shown to originate from e.g., “clock genes” that pleiotropically control circadian rhythm since the time of mating may cause temporal reproductive isolation (e.g., Miyatake et al. 2002). On the other hand, if mating between the species occurred post-mating isolation is likely to have reduced fertility of the hybrids (e.g., Munoz, Salazar, Castano, Jiggins and Linares 2010). All these reasons remain hypotheses until more empirical evidence on the differences in biology of these three sympatric *Macroplea* species is gathered.

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Legends

Figure 1. Map showing the collecting localities.

Figure 2. One of the authors (O. Biström) collecting *Macroplea* Samouelle, 1819 specimens in the field.

Figure 3. Strict consensus of the 20 most parsimonious trees resulting from TNT. Jackknife (JF) resampling values are shown on the nodes. Branch lengths represent the number of optimised character state changes.

Figure 4. Elytral spine: (a) *M. pubipennis* (Reuter, 1875) (Vaasa, Vaskiluoto); (b) *M. mutica* (Fabricius, 1792) (Finby, Laukalahti); (c) *M. mutica* (Estonia, Vormsi); (d) *M. appendiculata* (Panzer, 1794) (Vaasa, Vaskiluoto); (e) *M. appendiculata* (Kesälahti, Puruvesi); (f) *M. appendiculata* (Estonia, Maatsalu). Scale 1 mm.

Figure 5. Male metatarsomere: (a) *M. pubipennis* (Reuter, 1875) (Vaasa, Vaskiluoto): (b) *M. mutica* (Fabricius, 1792) (Finby, Laukalahti); (c) *M. mutica* (Estonia, Vormsi); (d) *M. appendiculata* (Panzer, 1794) (Kesälahti, Puruvesi); (e) *M. appendiculata* (Vaasa, Vaskiluoto). Scale 1 mm.

Figure 6. Pronotum: (a) *M. pubipennis* (Reuter, 1875) (Vaasa, Vaskiluoto); (b) *M. mutica* (Fabricius, 1792) (Estonia, Vormsi); (c) *M. appendiculata* (Panzer, 1794) (Vaasa, Vaskiluoto); (d) *M. appendiculata* (Kesälahti, Puruvesi). Scale 1 mm.

Figure 7. Dorsal habitus: (a) *M. pubipennis* (Reuter, 1875); (b) *M. appendiculata* (Panzer, 1794); (c) *M. mutica* (Fabricius, 1792).

Figure 8. Ventral habitus: (a) *M. pubipennis* (Reuter, 1875); (b) *M. appendiculata* (Panzer, 1794); (c) *M. mutica* (Fabricius, 1792).

Figure 9. Dorsal genital habitus: (a) *M. pubipennis* (Reuter, 1875); (b) *M. appendiculata* (Panzer, 1794); (c) *M. mutica* (Fabricius, 1792).

Figure 10. Lateral genital habitus: (a) *M. pubipennis* (Reuter, 1875); (b) *M. appendiculata* (Panzer, 1794); (c) *M. mutica* (Fabricius, 1792).

Table 1. Specimen data for the *Macroplea* Samouelle, 1819 used in this study.

Table 2. Uncorrected p-distances between the species calculated with MEGA v7.0.21. The analysis involved 208 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding and all positions containing gaps and missing data were eliminated. There were a total of 509 positions in the final dataset.

Table 3. List of plant species from which the *Macroplea* Samouelle, 1819 specimens were collected, in order of specimen abundance. Only the specimens whose host plant information is available are included.